A16

106. (New) Isolated muscle-derived progenitor cells obtained by the method according to claim 84.

REMARKS

In this amendment and response, the specification has been amended to correct spelling errors, such as the misspelling of the word "be" as mentioned by the Examiner on page 3 of the office action.

Claims 2, 27, 55, 94 and 95 have been cancelled without prejudice or disclaimer; claims 1, 84 and 96 have been amended; and claims 100-106 have been newly added. No new matter has been added by virtue of the amended and new claims which are supported by the claims and the application as filed. Specifically, support for long-term survivability or proliferation of the muscle-derived cells, and assessment in SCID (severe combined immune deficiency) mice in presently amended claim 1 is found in the instant specification, *inter alia*, at page 16, lines 13-14; at page 17, lines 8-10; at pages 35-36, Example 6 (MDC injected into bladder tissue had differentiated into myotubes or myofibers and extensive reinnervation had occurred after 6 months); at pages 49-50 (MDC differentiate into osteogenic cells in response to rhBMP-2 two weeks following injection into SCID mice); and also in FIGS. 15E-15G and 16A-16E. Support for amended claim 84 is found in the instant specification, *inter alia*, at page 14, lines 27-31; at page 15, lines 10-14; and at page 29, Example 1, e.g., lines 12-20. Support for the new claims is found in the originally filed specification and claims.

The amendments to the claims are presented for the purpose of expediting the patent application process in accordance with the PTO's Patent

Business Goals, 65 Fed. Reg. 54603 (September 8, 2000). The amendments to the specification and claims are found in the attached sheets entitled "Version with markings to show changes made". In the pages following the "Version with markings...", the currently pending claims are presented.

Claims 1, 3, 17-26, 45-54, 84, 92, 93, 96 and 100-106 are currently pending in this application. Applicants reserve the right to timely file one or more divisional applications covering the non-elected subject matter.

Information Disclosure Statement

The Examiner has informed applicants that the Information Disclosure Statement (IDS) filed on August 14, 2001 does not fully comply with the requirements of 37 C.F.R. §1.98 because "applicant does not properly cite the journal article(s) listed on the 1449. Misspelling of the word "intraarticular" in the title of reference no. 25."

To address this issue, applicants have provided herewith a new PTO 1449 form which properly cites the article in question. It is thus respectfully requested that the Examiner initial and date all of the references listed in the new PTO 1449 form, and return the initialed and dated PTO 1449 form to applicants with the next PTO communication.

35 U.S.C. §112, first paragraph

Claims 1-3, 17-27, 45-55, 84 and 92-96 have been rejected under 35 U.S.C. §112, first paragraph for the reasons set forth at pages 3-14 of the office action. According to the Examiner, the listed claims contain subject matter which was not described in such a way as to reasonably convey to one skilled in the relevant art that

the inventors had possession of the claimed invention at the time the application was filed.

Applicants respectfully traverse the rejection.

It is submitted that applicants' isolated muscle derived cells (MDC), while described from mouse, can be isolated using the preplating technique from other species and used as taught in the instant specification. Skeletal muscle of rats and mice are but two examples of suitable sources of the MDC according to the presently claimed invention; other sources include rabbits and humans. Such cells exhibit long-term (i.e., greater than about two weeks) survivability or proliferation after injection into muscle tissue of a recipient, and are found to be viable and to form muscle tissue in the transplanted and surrounding area for at least two weeks post-injection, as assessed by injection into a SCID mouse, or examination of the injected area of the recipient.

The isolated MDC described by applicants have the ability not only to survive for greater than about two weeks, but also to function as muscle tissue cells *in vivo*, following injection into a host animal. Applicants have described a means to assess whether isolated MDC are viable and proliferate and survive as claimed by injecting the cells into SCID mice and assessing whether the cells are viable muscle tissue cells at a time at least about two weeks after injection. MDC that possess these properties are within the metes and bounds of applicants' claimed invention.

Applicants have not only isolated muscle derived cells as described, they have demonstrated the use and function of the cells *in vivo* by injecting the cells into animal models in need of wound, injury, or surgical repair, for example, sphincter muscle severing, impaired bladder tissue, or a bone defect, or by injecting the cells into

different types of muscle tissue. The accompanying Declaration of Michael B. Chancellor under 37 C.F.R. §1.132 (¶¶ 9 a)-9 e)) describes the use of the presently claimed MDC in the disclosed treatments in art-accepted animal models.

As the Examiner has also recognized on page 7, last paragraph to page 8, lines 1-2 of the May 22, 2002 office action, applicants have provided numerous working examples in which the applicability of the isolated muscle cells has been demonstrated in more than one *in vivo* system. Both rats and mice show efficacy of treatment using the muscle derived cells newly discovered by the applicants. (e.g., "Examples 3-5 and 7-8 display that genetically modified MDC with LacZ were viable for up to 4 weeks in the lower abdomen of rats as shown in Example 3 (pages 32-33 and 39-40). Example 6 displays an increase in the contraction amplitude and contraction velocity (i.e., functional characteristics) of bladder strips of cryodamaged bladder tissue in rats using MDC (pages 33-39). Example 9 displays that genetically modified mc13 cells with adBMP-2 can cause bone formation (pages 40-51).

Experimental work based on the instant application, which was performed after the filing of the instant application and which follows and reproduces the teachings of the application, further demonstrate that MDC can be isolated not only from mice but also from rats and used as described in the instant specification. Both mouse and rat hosts have been shown to respond to injection of the isolated MDC by harboring viable muscle cells that function as muscle cells upon later investigation, i.e., over two weeks post injection. For example, the enclosed manuscript of the inventors and their technical laboratory researchers (J.Y. Lee et al., "The effects of periurethral muscle derived stem cell injection on leak point pressure in a rat model of stress urinary

incontinence", Exhibit 1) has been accepted for publication in 2002 in the journal *Urogynecology* and reports the successful use of rat MDC isolated as described in the instant application as a periurethral bulking agent to increase leak point pressure resulting from sphincter deficiencies in a denervated female rat model of stress urinary incontinence. In this accepted publication, it was found that the rat MDC injected into the denervated urethral sphincter muscle of anesthetized rats caused an increase in dorsolateral skeletal muscle masses with variable fiber orientation at the injection sites after four weeks. It was further shown that MDC isolated from rats as described by applicants survived in the lower urinary tract, developed into myofibrils to increase the presence of skeletal muscle fibers around the urethra and increased the leak point pressure in denervated rats following periurethral injection.

At page 9 of the office action the Examiner remarks that it is not apparent how an increase in the contractility of cryodamaged bladder tissue in rats..reasonably correlates to a method of treating weakness or dysfunction in muscle tissue ... using muscle derived cell therapy. Applicants point out that bladder and sphincter tissues, as exemplified in the application, are muscle tissues. Cryodamaged bladder tissue is representative of muscle tissue that has been injured or impaired so that it cannot properly or normally function. (See, Declaration of M. Chancellor, ¶ 8).

Applicants' MDC as presently claimed have been shown to be capable of repopulating an area of the bladder into which they were introduced and restoring function to such damaged bladder tissue (i.e., muscle tissue) following introduction or transplantation into the site of the damaged tissue. Indeed, numerous publications of

the inventors' own work, in collaboration with their technical laboratory researchers, and cumulative to the instant disclosure, support the long term survival of MDC and resulting myofiber formation in urethral and bladder wall (See, for example, T. Yokoyama et al., 2000, *World J. Urol.*, 18:56-61, Exhibit 2; T. Yokoyama et al., 2001, *J. Urol.*, 165:271-276, Exhibit 3; and J.Y. Lee et al., 2001, *J. Urol.*, 165(5), Supplement, 1033A:251, Exhibit 4).

The Examiner also remarks that applicants' exemplified rat model may not be considered standard in the art for reasonably extrapolating cell transplantation results in rats to other species and other types of muscle weakness and dysfunction. It is respectfully submitted that named inventor Michael Chancellor, M.D., and his laboratory and collaborators, have published numerous relevant studies in art-recognized and peer-reviewed journals in which rats, as an acceptable animal model system, have been used to test substances, e.g., collagen and fat, to treat urinary incontinence, with implications of the findings as providing beneficial treatments for muscle-related disorders or dysfunction in mammals other than rats. (See, the Declaration of M. Chancellor, ¶¶ 9 a)-9 e)) and the enclosed publication of M.E. Olson et al., 1998, *Urology*, 52:915-919, Exhibit 5). Applicants' development and use of MDC in accepted animal model systems provide an advance to the field, which is in need of new treatments for disorders and diseases associated with muscle tissue weakness, injury, or dysfunction.

In the field of stress urinary incontinence, which is a serious human problem, particularly in an aging population, rat model systems are routinely employed and accepted for studying and testing new treatment modalities and approaches. A

report by M.C. Heidkamp et al. (1998, *Int. Urogynecol. J.*, 9:88-93), provided herewith as Exhibit 6, shows that others in the field consider female rats to be an appropriate animal model, for example, to "allow preliminary hypothesis generation and testing prior to appropriately directed clinical trials" in connection with understanding the effects of various procedures and treatments and their applicability to humans.

In addition, the inventors are participating in collaborative and/or consulting relationships with industry in which the described animal models have been used to replicate augmentation of muscle tissue for the treatment of stress urinary incontinence in mammals other than the test animal models. More specifically, Dr. Chancellor is involved in ongoing research in this area with two known pharmaceutical companies, namely, Roche Pharmaceuticals – Palo Alto and Pfizer Pharmaceutical, Inc. (See, Declaration of M. Chancellor, ¶ 9d)). Dr. Chancellor has been working with these companies to study and test potential therapeutic agents to treat genitourinary disorders, such as urinary incontinence and/or bladder outlet obstruction, which involve muscle tissue weakness or dysfunction. The companies recognize and embrace studies that utilize female SD rats as appropriate models to extrapolate results of functional assays for incontinence and bladder outlet obstruction to other mammalian species, which have similar muscle-related weaknesses and disorders.

Pfizer, in collaboration with the work of Dr. Chancellor, has recognized that a rat model system of urinary incontinence, i.e., acutely spinal cord- / pudendal nerve-transected, urethane anesthetized female SD rats, provides both integrated and isolated manipulation of the internal and external urethral sphincters, which are contributors to continence. Also, this system can be combined with other

manipulations, such as pudendal nerve injury, hypogastric nerve injury, detrusor-sphincter dyssynergia, and the use of retired breeder rats as an approximation of pelvic floor weakness in multiparous human females, vaginal distension models and the like. Moreover, therapeutics can be functionally screened using this animal model of human pathological conditions involving muscle tissue.

Further, it is known and understood in the art that rats are recognized in the field as useful and practical animal models in which to perform *in vivo* studies for the purpose of extrapolating the results and findings to mammals other than rats. Indeed, the scientific and patent art is replete with publications disclosing the use of rats and other animal models as cell transplantation recipients with the goal of extrapolating the results to species other than the experimental animals actually used. The acceptability and common use of rats (and mice) as animal models of disease and as living tools to study treatments for broader applications in other mammals is further evidenced by the following exemplary publications, as provided herewith. The following representative publications support the art's recognition and acceptance of the animal (rat) models for testing and evaluating treatments for diseases and disorders, e.g., bladder defects, Huntington's Disease, and Parkinson's Disease, and extrapolating the obtained results to other species. The filing dates of the listed patents demonstrate that those in the art also recognized the use of animal models for cell transplantation at the time of filing the instant application.

1) O. Isacson et al., 1985, "Neural grafting in a rat model of Huntington's Disease: Progressive neurochemical changes after neostriatal ibotenate lesions and striatal tissue grafting", *Neuroscience*, 16(4):799-817. (Exhibit 7).

2) N. Nakao et al., 1995, "Overexpressing Cu/Zn superoxide dismutase enhances survival of transplanted neurons in a rat model of Parkinson's Disease", *Nature Medicine*, 1(3):226-231. (Exhibit 8).

3) A. Stenberg et al., 1999, "Injectable dextranomer-based implant: histopathology, volume changes and DNA analysis", *Scand. J. Urol. Nephrol.*, 33(6):355-361. (Exhibit 9).

4) U.S. Patent No. 6,254,865 B1 to C.R. Freed et al., filed June 17, 1998. (Exhibit 10).

5) U.S. Patent No. 6,060,048 to B.D. Cherksey, filed June 2, 1995. (Exhibit 11).

In view of the foregoing, the applicants submit that the rat model is art-recognized, appropriate and relevant for studies of muscle tissue disorder treatments and therapies using applicants' MDC, and that the results obtained from this model are extremely relevant for reasonably extrapolating effects and results to other mammalian species, including humans.

The Examiner mentions that the specification does not teach an "appropriate dose of muscle-derived cells per route of administration for a sustained and high enough level of expression of transplanted cells in any species". (office action, p. 9). Applicants disagree that no dose of cells is provided for use in the applications of the invention and point out that on page 20, lines 11-13 of the specification, it is disclosed that "...about 1-1.5 x 10^6 MDC are injected for the treatment of an approximately 8 mm diameter of cryodamage in bladder smooth muscle tissue...". In addition, at page 21, lines 5-6 of the specification, it is taught that "about

1-1.5 x 10^6 MDC are utilized for the augmentation of an approximately 5 mm region of the skin (see Example 3)". Page 22 also teaches this number of MDC for use in the various treatment applications of the invention. Also, Examples 3 through 8 of the application specifically teach that the skin or soft tissue of the animal was injected with an MDC suspension in HBSS (approximately 1-1.5 x 10^6 cells), and that this level of cells as starting material was able to improve function. It is also disclosed on page 18, lines 6-7 that for suspensions of MDC for administration to a subject, 10^8-10^9 cells/ml in a sterile solution can be used. In addition, as part of their routine knowledge, those having skill in the pertinent art also are able to determine doses for human testing based on testing and results obtained from animal models.

Thus, it is submitted that the instant specification does contain essential teachings that are specific to the administration of MDC, and that based on applicants' disclosure, teachings and exemplification, no undue amount of experimentation is required for one skilled in the art to reasonably extrapolate from the disclosure to the treatment of muscle dysfunction or weakness in a mammal.

The Examiner considers that the instant specification fails to "disclose assays demonstrating muscle function and to disclose that an increase in contractility of bladder strips from cryodamaged bladder tissue in rats, and the increased production of fibers is at a sufficiently high enough level so as to result in an increase in muscle tissue function"…. (office action, page 10). Applicants do not understand this reasoning, particularly in view of the entirety of the disclosure and Example 6, which

describes various aspects embracing the use of MDC in the treatment of cryodamaged bladder tissue.

Example 6 sets forth experimental data and results demonstrating that after 6 months following injection of MDC into rats in the amounts disclosed, extensive innervation was observed as evidenced by acetylcholine staining throughout the MDC-injected area of the bladder (page 36 of the specification). Also after 6 months, virtually all MDC had differentiated into myotubes or myofibers, as shown by an decrease in α-SM actin staining (Figure 5F), with a concomitant increase in fast MyHC staining (Figure 5I). Specific disclosure of contractility physiology studies performed on bladder samples from animals having varying degrees of injury in Example 6 reveals that MDC injections restored contractility to cryodamaged bladder muscle tissue, based on increased contraction amplitude and velocity values compared with control groups (Table 2, page 38). Thus, assays demonstrating muscle function are taught and the results indicate that MDC-based compositions can be utilized for the treatment of bladder injury or dysfunction which can be associated with urinary incontinence.

Bladder is a known muscle tissue, and contractility and velocity are measurable functions of muscle tissue as described. The restoration of contractility to damaged bladder muscle tissue (in which contractility is impaired) by the use of injected MDC in the rat model is a determination of effectiveness of the treatment. Example 6 of the instant application clearly describes, demonstrates and enables the restoration of muscle tissue function *in vivo* following damage to muscle tissue, and treatment by injection with the MDC as taught by applicants.

The Examiner also remarks that "it would be apparent to one skilled in the art that the addition of cells to the bladder would result in an increase of contraction amplitude due to the increase of muscle derived cells in the bladder strips". (page 9 of the office action). If this is believed to be so, then it is confusing to the applicants why the Examiner should allege that a determination of an increase in contraction amplitude and velocity as a result of treatment for damaged muscle with MDC is not an accepted parameter for correlating the effect of MDC injection to a therapeutic method of treating damaged bladder muscle, which should contract as part of its normal functioning.

Further, and importantly, applicants' invention does not involve a mere "addition of cells to the bladder", as if the bladder were an *ex vivo* entity, as implied by the Examiner. Rather, the invention involves the use of newly-described MDC as bioactive reagents for treating muscle injury, weakness, or dysfunction. MDC are not added to excised bladder; rather the isolated MDC are introduced *in vivo* into damaged bladder tissue as a muscle site in need of treatment or repair. It is an advantage and improvement newly provided to the art by the present invention that the MDC introduced into a muscle tissue, such as bladder or sphincter tissue, actually survive in the introduced area for long time periods, contrary to findings using other cell types, and have been shown to functionally assimilate in and around the muscle site without dying.

At page 10 of the office action, the Examiner specifically refers to claims 17-27, 45-55 and 92-93 and states that "one skilled in the art would reasonably determine that the cells could not be used in different species ... because of the

problem with exposing an animal to foreign cells (e.g. mouse cells) because of differences in the immune system of a mouse compared to another species (e.g. human, monkey, etc.)".

In this regard, the Examiner addresses xenogeneic or cross-species applications, while applicants disclose the autologous and allogeneic applicability of the disclosed MDC in muscle bulking and augmentation treatments. The Examiner remarks that the "as-filed specification does not teach how to prevent host vs. graft rejection and /or graft vs. host rejection disease (GVHD) in any mammal being treated with ... cells from a different species... for use in a different mammal". (office action, page 11). Applicants believe that concerns about rejection of transplanted cells by a host have been addressed in the instant specification, particularly at page 23, last paragraph to page 24, lines 1-3, where applicants teach that immunosuppressive agents can be used, as necessary, as can HLA (MHC) similarities between donor and recipient cells, or immunotolerization, as practiced in the art.

Thus, it is clear that applicants have described means of appropriately utilizing non-autologous MDC and have provided a teaching of ways to decrease potential problems with such cells. Further, applicants have determined that the MDC as described and provided by the presently claimed invention naturally possess a property of immune-privilege which contributes to their survival in transplanted tissue.

In the paper of J.Y. Lee et al., (Exhibit 1), improved transplantation capacity of rat-derived allogeneic MDC injected into female rats was observed one month post-injection. The transplanted MDC were found to be able to fuse with host muscle fibers, therefore allowing them to be better protected from an immune response

by the host. Other results from the study demonstrated that CD8 immune T cells were not present in the area injected with MDC compared with an area injected with non-MDC control cells. Thus, the improved transplantation capacity of non-autologous MDC can be at least partially be attributed to an absence of infiltration of activated CD8 lymphocytes at the site of MDC injection and to the lack of an immune response being triggered by the transplanted cells.

Applicants also note that embryonic porcine neural cells have been used to treat neurological defects due to neurodegeneration in xenogeneic hosts, as described in U.S. Patent No. 6,277,372 to T. Fraser et al., filed April 19, 1995. (Exhibit 12). Rejection by a host is often mooted by the immune privilege afforded by the naturally undifferentiated or immature state of embryonic or progenitor cells used as the agents of transplantation.

Accordingly, in view of the teaching of the specification, the immune-privileged nature of MDC in the treatments described, the knowledge of the skilled practitioner in the art pertaining to the use of immunosuppressive pre-treatments and treatments, as necessary, and the reports of other regimens involving embryonic cells of other species used in treating patients, it is submitted that the MDC as presently claimed would avoid rejection by a mammalian recipient's immune system following transplantation of the MDC into the recipient. Moreover, no undue or unreasonable amount of experimentation would be required to avoid transplantation rejection of the MDC by a recipient host, particularly in a non-autologous host, when immunosuppression of patients is routinely practiced in the art and such rejection is minimal to nil for the claimed MDC.

The Examiner has further remarked that the specification does not provide sufficient guidance for one skilled in the art to reasonably extrapolate the examples describing a method of augmenting or bulking bladder tissue because of the unpredictability of MDC differentiating into a specific muscle tissue and because of the differences between smooth and skeletal muscle in a mammal. It is submitted that applicants have taught and evidenced through examples that MDC obtained from skeletal muscle are fully capable of surviving and developing in smooth muscle tissue following introduction or transplantation into animals in an animal model system. The MDC exhibit long-term survivability and assimilate as muscle tissue cells for two weeks or longer post injection. Thus, it is believed that one skilled in the art would reasonably extrapolate the use of applicants' MDC in augmenting or bulking smooth muscle tissue for the types of treatment applications that are described by the applicants.

As further information that is cumulative to the originally filed disclosure, applicants provide two abstracts (Exhibits 13 and 14) reporting studies that were carried out based solely on the teachings of the instant application and as performed in the laboratories of the named inventors. The abstracts support the teachings of applicants' disclosure (e.g., Example 7, page 39) and demonstrate that skeletal muscle derived MDC exhibit long term survival (e.g., for 8 weeks and more) and associate with heart muscle (non-skeletal muscle) components following transplantation into heart muscle (myocardium).

1. H. Oshima et al., 2002, "Long-term survival of novel muscle-derived stem cells after transplantation into myocardium", 10th International Congress on Neuromuscular Disease, July 7-12, 2002, Vancouver, Canada. (Exhibit 13).

2. T. Payne et al., 2002, "Novel muscle-derived stem cells deliver dystrophin into a dystrophin-deficient murine heart", 10th International Congress on Neuromuscular Disease, July 7-12, 2002, Vancouver, Canada. (Exhibit 14).

Another abstract, B. Cao et al., 2002, "Muscle stem cells differentiate into hematopoietic lineage but retain myogenic potential", 10th International Congress on Neuromuscular Disease, July 7-12, 2002, Vancouver, Canada, (Exhibit 15) reports on the ability of MDC to disseminate and restore dystrophin in the gastrocnemius muscles of injected irradiated recipient adult mdx mice, and that these cells also possessed long-term repopulating capacity and plasticity in being able to differentiate into hematopoietic lineage cells. That such studies based on the teachings of applicants' disclosure are currently ongoing offers support both for the actual practice of the presently claimed invention and for the benefits afforded by the disclosed MDC-based treatments and therapies.

In light of the foregoing explanation and discussion, it is believed that enablement is clearly and reasonably provided for the entire breadth of the presently claimed invention. Accordingly, withdrawal of the 35 U.S.C. §112, first paragraph rejection is respectfully requested.

35 U.S.C. §112, second paragraph

Claims 1, 84 and 94 have been rejected under 35 U.S.C. §112, second paragraph, as being indefinite for the reasons given on page 14 of the office action.

Applicants respectfully traverse the rejection and submit that the presently amended claims address the remarks of the Examiner concerning these claims and

overcome the §112, second paragraph, rejection. The amendments to the claims serve to advance the progress of this application to allowance, without acquiescing to the propriety of the rejection, or to the contention that the claims are indefinite pursuant to Section 112, second paragraph. It is respectfully believed that all of the presently pending claims satisfy the requirements of 35 U.S.C. § 112, second paragraph. Accordingly, reconsideration and withdrawal of the §112, second paragraph rejection are respectfully requested.

Double Patenting

Claims 1, 3, 84 and 94-95 have been provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 76-80 of co-pending application U.S. Serial No. 09/302,896. According to the Examiner, the conflicting claims are not identical, but are considered to be obvious variants of one another. It is submitted that applicants will address this rejection by the filing of a terminal disclaimer upon indication of allowability of subject matter in the instant application.

35 U.S.C. § 103

Claims 1, 3, 84 and 94-95 have been provisionally rejected under 35 U.S.C. § 103 (a) as being obvious over co-pending application U.S. Serial No. 09/302,896, which has a common inventor with the instant application. The Examiner has based this rejection n a presumption of future publication or patenting of the conflicting application.

Applicants respectfully traverse this rejection. It is submitted that the instant application was filed on April 14, 2000, which is after the November 29, 1999 date. The subject matter of the claims of the instant application and the subject matter of the reference were, at the time the invention was made, commonly-owned by the same entity. The instant application is assigned to The University of Pittsburgh (recordation date: August 7, 2000) and application U.S. Serial No. 09/302,896 is also assigned to the same entity (recordation date: June 28, 1999). The two applications name common inventors. Thus, it is requested that this rejection be reconsidered and withdrawn.

AUTHORIZATION

Should any additional fees be deemed to be properly assessable in this application for the timely consideration of this amendment and response, the Commissioner is hereby authorized to charge any such additional fee(s), or to credit any overpayment, to Deposit Account No. 13-4500, Order No. 2710-4007US2. **A duplicate copy of this sheet is attached.**

CONCLUSION

In the event that the Examiner is of the opinion that further discussion of the application would be helpful, the Examiner is hereby respectfully requested to telephone the applicants' undersigned representative at (212) 415-8751 and is assured of full cooperation in an effort to advance the prosecution of the instant application and

claims to allowance.

Respectfully submitted,

MORGAN & FINNEGAN, L.L.P.

Date: November 21, 2002

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Version with Markings to Show Changes Made

In the Specification

At page 19, the paragraph at lines 15-27 has been replaced as follows:

To optimize transplant success, the closest possible immunological match between donor and recipient is desired. If an autologous source is not available, donor and recipient Class I and Class II histocompatibility antigens can [0be] be analyzed to determine the closest match available. This minimizes or eliminates immune rejection and reduces the need for immunosuppressive or immunomodulatory therapy. If required, immunosuppressive or immunomodulatory therapy can be started before, during, and/or after the transplant procedure. For example, cyclosporin A or other immunosuppressive drugs can be administered to the transplant recipient. Immunological tolerance may also be induced prior to transplantation by alternative methods known in the art (D.J. Watt et al., 1984, *Clin. Exp. Immunol.* **55**:419; D. Faustman et al., 1991, *Science* **252**:1701).

At page 33, description of Example 5, the paragraph at lines 8-18 has been replaced as follows:

SD rats were prepared for surgery as described above. A midline abdomen incision was made to expose the ureteral-bladder (vesico-ureteral) junction. The tissue was injected with 10 μl of MDC suspension in HBSS (1-1.5 x 10^6 cells) using a Hamilton microsyringe. At 3 days post-injection, the area surrounding each injection site was excised, prepared for histological analysis, stained for β-galactosidase to determine the location and viability of the cells carrying the LacZ marker, examined microscopically, and photographed. These results demonstrate that MDC-based compositions can be used as utereral-bladder augmentation materials (Figures 3A and 3B) for the treatment of [vesico-utereal] vesico-ureteral reflux symptoms or conditions.

In the Claims

Cancelled claims:

Claims 2, 27, 55, 94 and 95 have been cancelled without prejudice or disclaimer.

Amended claims:

Claims 1, 84 and 96 have been amended as follows:

1. (Amended) Isolated mammalian muscle-derived progenitor cells expressing desmin as a cell surface protein and having long-term survivability when introduced into [mammals] an autologous or allogeneic mammalian recipient host, wherein long term survivability is determined by viability or proliferation of the cells as muscle tissue cells at or near a site of introduction for greater than or equal to about two weeks following subcutaneous injection into (i) a severe combined immune deficient (SCID) mouse model system or (ii) the recipient host [the cells express cell markers selected from the group consisting of at least desmin, CD34, and Bcl-2].

84. (Amended) A method of isolating [the] muscle-derived progenitor cells from a mammal, [according to claim 1], comprising:

a. enzymatically digesting muscle tissue to obtain a suspension of cells;

b. plating the cell suspension in a collagen coated [flask] container;

c. removing the suspended, non-adherent cells;

d. re-plating the cells of (c) in a second collagen coated [flask] container;

e. repeating steps (c) and (d) thereby enriching for viable, slowly adhering cells in the container, wherein a last plating comprises the viable, slowly adhering cells and virtually no fibroblast cells [at least five times]; and

f. isolating the viable, slowly adhering cells present after the last plating.

96. (Amended) The [clonally isolated] cells according to [claim 94] claim 1 or claim 104, [further] wherein the cells co-express at least one cell marker selected from the group consisting of CD34, Bcl-2, Sca-1 and Flk-1 [cell markers], and do not express CD45 and c-Kit cell markers.

Currently Pending Claims (USSN: 09/549,937)

1. (Amended) Isolated mammalian muscle-derived progenitor cells expressing desmin as a cell surface protein and having long-term survivability when introduced into an autologous or allogeneic mammalian recipient host, wherein long term survivability is determined by viability or proliferation of the cells as muscle tissue cells at or near a site of introduction for greater than or equal to about two weeks following subcutaneous injection into (i) a severe combined immune deficient (SCID) mouse model system or (ii) the recipient host.

3. (Unamended) A physiologically acceptable composition comprising the muscle-derived progenitor cells according to claim 1, and a carrier, excipient, or diluent.

17. (Unamended) A method of augmenting or bulking muscle tissue in a mammal comprising: administering the composition according to claim 3 to the muscle tissue in an amount sufficient to augment or bulk the tissue.

18. (Unamended) The method according to claim 17, wherein the muscle tissue is skeletal or smooth muscle tissue.

19. (Unamended) The method according to claim 17, wherein the muscle tissue is selected from the group consisting of digestive, reproductive, cardiovascular, urological, and respiratory tissue.

20. (Unamended) The method according to claim 19, wherein the digestive tissue is selected from the group consisting of tongue, esophageal, stomach, intestinal, and anal tissue.

21. (Unamended) The method according to claim 19, wherein the reproductive tissue is selected from the group uterine, vaginal, clitoral, fallopian tubes, penile, and vas deferens tissue.

22. (Unamended) The method according to claim 19, wherein the cardiovascular tissue is selected from the group consisting of arteries, capillaries, veins, and heart tissue.

23. (Unamended) The method according to claim 19, wherein the urological tissue is selected from the group consisting of kidney, bladder, urethral, and ureter tissue.

24. (Unamended) The method according to claim 19, wherein the respiratory tissue is tracheal or lung tissue.

25. (Unamended) The method according to claim 17, wherein the administration is by injection into the muscle tissue or by intravenous delivery.

26. (Unamended) The method according to claim 17, wherein the administration is mediated by an absorbent or adherent carrier.

45. (Unamended) A method of treating weakness or dysfunction in muscle tissue in a mammal comprising: administering the composition according to claim 3 to the muscle tissue in amounts sufficient to treat the weakness or dysfunction.

46. (Unamended) The method according to claim 45, wherein the tissue is skeletal or smooth muscle tissue.

47. (Unamended) The method according to claim 45, wherein the weakness or dysfunction is secondary to a sports-related injury.

48. (Unamended) The method according to claim 46, wherein the tissue is sphincter tissue.

49. (Unamended) The method according to claim 46, wherein the tissue is selected from the group consisting of esophageal, anal, cardiac, pyloric, and urinary sphincter tissue.

50. (Unamended) The method according to claim 45, wherein the weakness or dysfunction is selected from the group consisting of vesico-ureteral reflux, urinary incontinence, gastroesophageal reflux, and fecal incontinence.

51. (Unamended) The method according to claim 45, wherein the tissue is heart tissue.

52. (Unamended) The method according to claim 51, wherein the weakness or dysfunction is secondary to heart failure or myocardial infarction.

53. (Unamended) The method according to claim 45, wherein the administration is by injection into the tissue or by intravenous delivery.

54. (Unamended) The method according to claim 45, wherein the administration is by an absorbent or adherent material.

84. (Amended) A method of isolating muscle-derived progenitor cells from an mammal, comprising:

a. enzymatically digesting muscle tissue to obtain a suspension of cells;

b. plating the cell suspension in a collagen coated container;

c. removing the suspended, non-adherent cells;

d. re-plating the cells of (c) in a second collagen coated container;

e. repeating steps (c) and (d) thereby enriching for viable, slowly adhering cells in the container, wherein a last plating comprises the viable, slowly adhering cells and virtually no fibroblast cells; and

f. isolating the viable, slowly adhering cells present after the last plating.

92. (Unamended) A method of treating aesthetic or cosmetic defects, comprising administering subcutaneously or intradermally the composition according to claim 3.

93. (Unamended) The method according to claim 92, wherein the composition is administered by injection into the tissue.

96. (Amended) The cells according to claim 1 or claim 104, wherein the cells co-express at least one cell marker selected from the group consisting of CD34, Bcl-2, Sca-1 and Flk-1, and do not express CD45 and c-Kit cell markers.

100. (New) The method according to claim 84, wherein the muscle tissue of (a) is skeletal muscle.

101. (New) The method according to claim 84, wherein steps (c) and (d) are repeated at least five times.

102. (New) A method of augmenting or bulking muscle tissue in a mammalian host, comprising: administering a physiologically acceptable composition comprising desmin-expressing autologous or allogeneic muscle derived progenitor cells, characterized in that the cells survive or proliferate as muscle tissue cells in and around a site of administration for at least about two weeks following injection into the host, wherein the cells are administered to the host in an amount sufficient to augment or bulk the muscle tissue.

103. (New) A method of treating weakness or dysfunction in muscle tissue in a mammalian host, comprising: administering a physiologically acceptable composition comprising desmin-expressing autologous or allogeneic muscle derived progenitor cells, said cells characterized in that the cells survive or proliferate as muscle tissue cells in and around a site of administration for at least about two weeks following injection into the host, wherein the cells are administered to the host in an amount sufficient to treat the muscle tissue weakness or dysfunction.

104. (New) An isolated population of desmin-expressing mammalian muscle-derived progenitor cells having functional long-term survivability when introduced into a mammalian recipient host, wherein functional long term survivability is determined by viability or proliferation and function of the cells as muscle tissue cells for greater than or equal to two weeks at or near a site of introduction following subcutaneous injection into a severe combined immune deficient (SCID) mouse model system or into the recipient host.

105. (New) A clonal population of cells further isolated from the cells according to claim 1 or claim 104.

106. (New) Isolated muscle-derived progenitor cells obtained by the method according to claim 84.

Applicants respectfully traverse this rejection. It is submitted that the instant application was filed on April 14, 2000, which is after the November 29, 1999 date. The subject matter of the claims of the instant application and the subject matter of the reference were, at the time the invention was made, commonly-owned by the same entity. The instant application is assigned to The University of Pittsburgh (recordation date: August 7, 2000) and application U.S. Serial No. 09/302,896 is also assigned to the same entity (recordation date: June 28, 1999). The two applications name common inventors. Thus, it is requested that this rejection be reconsidered and withdrawn.

AUTHORIZATION

Should any additional fees be deemed to be properly assessable in this application for the timely consideration of this amendment and response, the Commissioner is hereby authorized to charge any such additional fee(s), or to credit any overpayment, to Deposit Account No. 13-4500, Order No. 2710-4007US2. **A duplicate copy of this sheet is attached.**

CONCLUSION

In the event that the Examiner is of the opinion that further discussion of the application would be helpful, the Examiner is hereby respectfully requested to telephone the applicants' undersigned representative at (212) 415-8751 and is assured of full cooperation in an effort to advance the prosecution of the instant application and